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(54) Title: METHOD OF TREATING MUSCULAR DYSTROPHY

(57) Abstract

This invention relates to a method of treating muscle disorders, such as muscular dystrophy, which demonstrate a decrease in muscle protein synthesis, an increase in protein degradation or both. The treatment comprises the administration of an effective dose of insulin-like growth factor I ("IGF-I") or genetically engineered human IGF-I (hIGF-I) to a mammalian subject. The invention also includes a method of IGF-I or hIGF-I treatment combined with a high protein diet and/or exercise regimen to further IGF-I or hIGF-I related improvement.

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METHOD OF TREATING MUSCULAR DYSTROPHY

FIELD OF THE INVENTION

This invention relates to the development of therapies for the treatment of muscle disorders, such as muscular dystrophy, which demonstrate a decrease in muscle protein synthesis, an increase in protein degradation or both.

BACKGROUND OF THE INVENTION

The term muscular dystrophy (MD) encompasses a number of devastatingly progressive muscle diseases for which no effective treatment is known. MD is found in many species of mammals, including livestock, dogs, hamsters, mice, chicken, as well as humans.

The major manifestations of MD diseases are progressive skeletal muscle weakness and wasting. It is thought that the progressive muscle weakness and wasting are at least partly caused by an imbalance between muscle protein synthesis and protein degradation. It is further thought that this imbalance results from a decrease in muscle protein synthesis and a disturbance in amino acid metabolism both of which are seen in such MD diseases as myotonic dystrophy, acid maltase deficiency, limb girdle dystrophy, nemaline myopathy, fascio-scapular humoral dystrophy, Becker muscular dystrophy and Duchenne muscular dystrophy. Under one theory the decrease in muscle protein synthesis is believed to be caused by an impaired

end-organ response to anabolic hormones or substrates. Under another theory, the decrease in muscle protein synthesis is believed to result from a disturbance in amino acid metabolism. The resultant decrease in available amino acids causes the muscles to be deprived of the amino acids needed for protein synthesis.

One hypothesis advanced is that the impaired end-organ response may be caused by a resistance to the action of insulin. However, no clear mechanism for such an insulin resistance has been reported. Based on abnormalities found in the plasma membrane of mammals with MD, some believe that a resistance to insulin occurs at the level of the plasma membrane receptor, particularly the skeletal muscle receptor. This resistance to insulin is manifested by problems with insulin receptors on monocytes and fibroblasts. Still others believe that insulin resistance is caused by post receptor dysfunction

To date, however, no one theory has been proven true and no cure for MD has been found. The currently available treatments for slowing the progression of muscle weakness and wasting, such as the use of prednisone for treatment of Duchenne MD, are only minimally effective.

There have been a number of attempts to enhance muscle protein synthesis as a means of delaying the progressive muscle deterioration associated with myotonic dystrophy, including the use of anabolic steroids, testosterone and high doses of insulin. From animal studies, the three known ways of enhancing

muscle protein synthesis and decreasing protein degradation are: increasing the availability of circulating amino acids, increasing the frequency of muscle contractions, and increasing the presence of adequate circulating insulin.

Some success has been achieved through efforts to increase the availability of circulating amino acids and increase the frequency of muscle contractions. For instance, the administration of a combination of a high protein diet along with a submaximal exercise therapy (HPET) has been moderately successful in slowing the deterioration of dystrophic muscles. However, the effect is limited, and is only useful if started early in the disease process. Once wasting is present, HPET does not appear to be effective.

Efforts to increase protein synthesis, such as administering testosterone or administering supraphysiological concentrations of insulin have proven unsuccessful. Thus, a means to increase insulin utilization remains a problem, and has lead to a variety of theories regarding possible generalized insulin resistance in mammals having MD.

SUMMARY OF THE INVENTION

In light of the foregoing, it is an object of the present invention to develop methods and therapies for the effective treatment of mammals having MD. It also is an object of this invention to slow or stop the deterioration of muscle associated with MD. It is a further object of the present

invention to improve the anabolic, metabolic, and clinical response of mammals having MD. Finally, it is an object of the present invention to overcome the apparent insulin resistant state of muscle in mammals having MD.

These, and the other objects of the invention are achieved through administration of either insulin-like growth factor I (IGF-I), or genetically engineered human IGF-I (hIGF-I), to a mammalian subject having MD. The invention further includes a method of hIGF-I treatment in combination with a diet and/or exercise regimen to further hIGF-I related improvement.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1(A) shows the change in endurance of mice of Example B over the course of treatment with hIGF, hIGF and high protein diet or placebo compared to a non-dystrophic mouse control;

Figure 1(B) shows the change in limb flexibility of mice of Example B over the course of treatment with hIGF, hIGF and high protein diet or placebo compared to a non-dystrophic mouse control;

Figure 2(A) shows the change in the content of RNA in soleus muscle of mice of Example B over the course of treatment with hIGF, hIGF and high protein diet or placebo, and a non-dystrophic mouse control;

Figure 2(B) shows the change in the content of RNA in extensor digitorum longus ("EDL") muscle of mice from Example B

over the course of treatment with hIGF, hIGF and high protein diet or placebo, and a non-dystrophic mouse control;

Figure 2(C) shows the change in the content of RNA in gastrocnemius muscle of mice from Example B over the course of treatment with hIGF, hIGF and high protein diet or placebo, and a non-dystrophic mouse control;

Figure 3(A) shows the change in the content of DNA in soleus muscle of mice from Example B over the course of treatment with hIGF, hIGF and high protein diet or placebo, and a non-dystrophic mouse control;

Figure 3(B) shows the change in the content of DNA in EDL muscle of mice from Example B over the course of treatment with hIGF, hIGF and high protein diet or placebo, and a non-dystrophic mouse control;

Figure 3(C) shows the change in the content of DNA in gastrocnemius muscle of mice from Example B over the course of treatment with hIGF, hIGF and high protein diet or placebo, and a non-dystrophic mouse control;

Figure 4(A) shows the change in the rate of protein synthesis, determined using a phenylalanine marker, in soleus muscle of mice from Example B over the course of treatment with hIGF, hIGF and high protein diet or placebo, and a non-dystrophic mouse control;

Figure 4(B) shows the change in the rate of protein synthesis, determined using a phenylalanine marker, in EDL muscle of mice from Example B over the course of treatment with

hIGF, hIGF and high protein diet or placebo, and a non-dystrophic mouse control;

Figure 4(C) shows the change in the rate of protein synthesis, determined using a phenylalanine marker, in gastrocnemius muscle of mice from Example B over the course of treatment with hIGF, hIGF and high protein diet or placebo, and a non-dystrophic mouse control;

Figure 5(A) shows the change in protein degradation, as measured by urinary 3-methylhistidine excretion, per unit body weight of mice from Example B over the course of treatment with hIGF, hIGF and high protein diet or placebo, and a non-dystrophic mouse control;

Figure 5(B) shows the change in protein degradation, as measured by urinary 3-methylhistidine excretion, per mg creatinine of mice from Example B over the course of treatment with hIGF, hIGF and high protein diet or placebo, and a non-dystrophic mouse control;

Figure 5(C) shows the change in protein degration, as measured by urinary 3-methylhistidine excretion, per volume urine of mice from Example B over the course of treatment with hIGF, hIGF and high protein diet or placebo, and a non-dystrophic mouse control;

Figure 6(A) shows the change in protein content of soleus muscle of mice from Example B over the course of treatment with hIGF, hIGF and high protein diet or placebo, and a non-dystrophic mouse control;

Figure 6(B) shows the change in protein content of EDL muscle of mice from Example B over the course of treatment with hIGF, hIGF and high protein diet or placebo, and a non-dystrophic mouse control;

Figure 6(C) shows the change in protein content of gastrocnemius muscle of mice from Example B over the course of treatment with hIGF, hIGF and high protein diet or placebo, and a non-dystrophic mouse control;

Figure 7(A) is a photomicrograph of muscle fiber from a dystrophic mouse;

Figure 7(B) is a photomicrograph of muscle fiber from a dystrophic mouse fed a high protein diet;

Figure 7(C) is a photomicrograph of muscle fiber from a non-dystrophic mouse; and

Figure 7(D) is a photomicrograph of muscle fiber from a dystrophic mouse given hIGF-1 treatment.

DETAILED DESCRIPTION OF THE INVENTION

Research carried out on various MD diseases has consistently revealed a disturbed amino acid metabolism, along with a decrease in muscle protein synthesis. It is thought that this decrease in muscle protein synthesis causes an imbalance between muscle protein synthesis and protein degradation, which in turn leads to progressive muscle weakness and wasting. It is further thought that the decrease in muscle protein synthesis found in MD may be caused by an impaired end-organ response to

anabolic hormones resulting in decreased uptake of substrate by muscle.

Surprisingly, I have now obtained pharmacologic effects with a resultant increase in muscle synthesis and decrease in muscle degradation in vivo in animals and humans through the administration of hIGF-I. In vivo, hIGF-I displays potent insulin-like activity in target organs for insulin, especially muscle. In isolated muscles, hIGF-I causes an increase in the uptake of glucose and amino acids. As a result, hIGF-I is able to enhance muscle protein synthesis and possibly overcome insulin resistance, resulting in an improvement in overall muscle strength and muscle function in mammals having MD.

Examples of parameters useful in monitoring improvement in human subjects resulting from administration of hIGF-I include 1) serum creatine kinase, which is typically elevated in "active myopathies" (note that in myotonic dystrophy it is often normal), 2) muscle biopsy findings which reveal atrophy or necrosis of muscle fibers, 3) 3-methylhistidine excretion, an index of muscle protein degradation is increased, (note that in myotonic dystrophy this parameter is normal), and 4) the rates of muscle protein synthesis as measured by whole body ^{13}C-leucine turnover and ^{13}C-leucine incorporation into muscle protein are mildly and markedly decreased, respectively, in MD. Other tests such as the stable-label intravenous glucose tolerance test ("IVGTT") minimal model to determine the effect

of hIGF-I on glucose metabolism, and tests to determine muscle strength and function such as hand-grip dynamometry, manual muscle testing and Cybex® isokinetic dynamometry and timed function tests may prove equally effective in determining whether clinical improvement has occurred.

For other mammals, further tests for measuring the amount of amino acid incorporated into specific muscles may be useful in determining muscle protein synthesis and protein degradation. An appropriate method to determine the amount of amino acid incorporated for mammals such as hamsters, is by the hemicorpus perfusion of the lower limbs, particularly the soleus and quadriceps muscles, using tritiated phenylalanine. Phenylalanine is chosen as a suitable marker for the determination of protein synthesis because it is neither synthesized nor degraded by muscle. However, other markers such as tyrosine could be used as well.

The rate of protein synthesis is determined from the ratio of radioactivity incorporated per milligram of muscle protein and the specific radioactivity of intracellular free phenylalanine. Muscle phenylalanine content is determined fluorometrically. The extracellular spaces are determined in separate muscle preparations by incubation with ^{3}H Inulin. Protein degradation is determined by the dilution of ^{14}C phenylalanine by ^{12}C phenylalanine.

Morphologic parameters for mammals such as mice can be determined by such means as volume density and shape factor of muscle fibers. One example of such an evaluation for mice involves examination of samples of soleus (red or type I fibers) and quadriceps (white or type IIB fibers) muscles by light and electron microscopy. For light microscopy, the percent of necrotic fibers, abnormalities in the distribution of fiber sizes, inflammatory cell number and changes in number of satellite cells are determined. For electron microscopy, plasma membrane alterations (gaps or disappearance of the sarcolemma), basal lamina replication, mitochondrial swelling or degeneration, decreases in myofilament number and fiber rarefaction, hypercontraction of fibers, ghost fibers and dilation of sarcoplasmic reticulum are determined.

The examples which follow are purely illustrative and not intended to limit the breadth of the invention disclosed herein.

EXAMPLE

Example A - human test

Human subjects having myotonic dystrophy are admitted to a hospital for a period of 7 days. On the third hospital day, the subjects undergo two successive stable label studies. The first of these studies is performed in the morning after a 10 hour overnight fast. Initially, a stable-label IVGTT minimal

model lasting 240 minutes measuring insulin sensitivity and glucose effectiveness is performed. The second study, which is performed in the fed state, is a primed continuous 150 minute infusion of L-[1-^{13}C]leucine for measuring whole body leucine flux, leucine oxidation, non-oxidative leucine disposal, the latter being an index of the rate of leucine incorporation into body protein (protein synthesis). Beginning 1 hour before the leucine infusion, and hourly thereafter, the patients consume small isocaloric, isonitrogenous meals, each equivalent to one-twelfth of their total daily protein and energy intake. Starting on the morning of the fourth hospital day, the subject receives either an identical placebo, or recombinently produced hIGF-I obtained from Genentech. The dose is administered twice a day by means of subcutaneous route. The dose strength ranges from 0.06 to 0.12 mg/kg dose and is designed to produce an anabolic effect, yet not cause hypoglycemia. As hIGF-I is administered subcutaneously, the period between dose injection and meal intake is at least 30 minutes.

During the first day of hIGF-I administration the subject's blood glucose is monitored every 2 hours, and over the succeeding 3 days the subject's blood glucose is monitored every 4 hours, to ensure that hypoglycemia (blood glucose <60 mg/dl) does not occur. If the prescribed dose of hIGF-I causes hypoglycemia, the dose is reduced to 0.06 mg/dl or a lower dose, to prevent hypoglycemia.

Improvement is determined by measuring the effect of hIGF-I on glucose metabolism, using the stable-label IVGTT minimal model and the effect of hIGF-I on amino acid metabolism, using the rates of leucine turnover, oxidation and incorporation into protein. Clinical response is determined by measuring change in muscle strength, muscle function and by studying body composition. These parameters are monitored throughout treatment and based on their results, adjustments are made to the subjects' dose, diet and/or exercise regiment. It should be noted that other parameters such as total body potassium [^{40}K] could also be used.

The effect of hIGF-I on glucose and amino acid metabolism is measured at the conclusion of 4 months of hIGF-I therapy. The effect of hIGF-I on glucose metabolism is assessed by the stable-label IVGTT minimal model using [6,6-$^{2}D_2$] glucose as label. The effect of hIGF-I on amino acid metabolism is assessed by measuring rates of leucine turnover, oxidation and incorporation into protein utilizing a primed, continuous infusion of the stable isotope L-[1-^{13}C]leucine.

Evidence indicative of improvement is determined by analyzing serum creatine kinase, muscle strength and function, 3-methylhistidine excretion, glucose metabolism as determined using the stable label IVGTT test, and the rates of whole body and muscle protein synthesis as measured by whole body ^{13}C-

leucine turnover and ^{14}C-leucine incorporation into muscle protein.

To determine the effect on muscle function and body composition, paired muscle strength and muscle function examination is performed by a physical therapist prior to starting therapy and again when treatment is discontinued. Single muscle strength and muscle function evaluations are performed monthly after therapy is started. Muscle strength is evaluated by manual muscle testing, hand-grip dynamometry and Cybex® isokinetic dynamometry. Muscle function is evaluated by timed functional testing. Pulmonary function is measured by routine spirometry. Muscle mass is evaluated by creatinine excretion and lean body mass by measurement of bioelectrical impedance technique, and dual-energy x-ray absorptiometry (DXA).

Example B - Mouse

A test was set up using two separate groups of mice; dystrophic and control mice. Twelve genetically dystrophic mice (129 B6F/J dy) were used as the animal model of choice. These animals exhibited the muscle weakness and wasting characteristics of muscular dystrophy. An insulin resistance was also present. Twelve control animals were of the same strain but lacking in the genetic defect (129/J). The IGF-I was administered subcutaneously in a dose of 1 mg/kg. Phosphate buffered saline was injected as placebo in mice not receiving

IGF-I. The recombinant hIGF-I administered to the mice was supplied by Genentech.

Dystrophic mice (4-6 weeks of age) were randomly assigned to groups I-IV, and age and sex matched controls were assigned to group V. Dystrophic animals from group I received a diet of normal mouse chow having a protein content of 20% and placebo. Dystrophic animals in group II received subcutaneous hIGF-I and were fed normal mouse chow. Dystrophic animals in group III received subcutaneous hIGF-I and were fed a high protein diet having a protein content of 50%. Dystrophic animals in group IV received subcutaneous hIGF-I along with a high protein diet. Control animals in group V received a diet of normal mouse chow and placebo.

All five groups continued on their respective protocols for four weeks. The animals had free access to both food and water. Blood for glucose and amino acids was drawn under light CO_2 anesthesia from the suborbital sinus.

Baseline measurements of body length, body mass, muscle strength, serum amino acid and blood glucose concentrations were made in all groups of animals. Body weights and lengths, and blood glucose levels were measured twice weekly throughout the study.

For each mouse, a twice weekly measurement was taken of its length from nose to tail and body weight. Once a week, muscle strength was determined by subjectively rating the animal's hind-limb utilization on a scale of 1 to 5 (1 being an

animal that dragged its hind limbs and pulled itself forward with its forelimbs and 5 being an animal that fully used its hind limbs). Also once a week, endurance were determined by measuring the length of time an animal remained suspended from a wire rack 12 inches above the tabletop. Blood was obtained at the beginning and end of the study for glucose, amino acids and recombinant hIGF-I. Also measured was the rate of protein synthesis which was determined by measuring the ratio of radioactivity incorporated per milligram of muscle protein and the specific radioactivity of intracellular free phenylalanine.

Morphological parameters also were examined to determine improvement based on treatment. To accomplish this examples of soleus (red or type I fibers) and quadriceps (white or type IIB fibers) muscles were examined by light and electron microscopy. For examination with light microscopy, the percent of necrotic fibers, abnormalities in the distribution of fiber sizes, inflammatory cell number and changes in number of satellite cells were determined. For examination with electron microscopy the plasma membrane alterations (gaps or disappearance of the sarcolemma), basal lamina replication, mitochondrial swelling or degeneration, decreases in myofilament number and fiber rarefaction, hypercontraction of fibers, ghost fibers and dilation of sarcoplasmic reticulum were determined. These light and electron microscopy findings were characteristic of the muscle degeneration which occurs in MD.

The increased protein synthesis achieved through administration of hIGF-I was reflected by the enhanced amino acid uptake and increased levels of muscle protein, amino acids, RNA and DNA and an overall preservation of muscle mass and muscle function. Furthermore, the effectiveness of hIGF-I was believed to have been potentiated by co-administration of a high protein diet which increased the amino acid availability.

The following tables report the results of several measurements made on muscle removed from the animals after 4 weeks. The data labeled NPD were obtained from dystrophic control mice, the data labeled IGF were obtained from dystrophic mice given hIGF-1, the data labeled IGF+HPD were obtained from dystrophic mice given hIGF-1 and a high protein diet, and the data labeled CONT were obtained from non-dystrophic control mice. The label SOL identifies muscle fiber from the soleus, the label EDL identifies muscle fiber from the extensor digitus longus, and the label GAST identifies muscle fiber from the gastrocenemius. In these tables, the data with a single astric (*) are significantly different from that of the untreated dystrophic mice at the 0.05 level and the data with a double astric (**) are significantly different from that of the untreated dystrophic mice at the 0.01 level.

Table 1: Protein Synthesis ($pmol^{-1h}$/mg muscle)

	NPD	IGF	IGF+HPD	CONT
SOL	29.6 ±3.0	32.8 ±2.5	47.9 ±4.8**	20.4 ±2.0
EDL	25.5 ±3.1	31.9 ±3.1	34.5 ±5.5*	15.5 ±1.8*
GAST	18.9 ±2.1	25.8 ±2.5	29.1 ±3.7*	5.75 ±.66**

Table 2: Protein Content (ug/mg muscle)

	NPD	IGF	IGF+HPD	CONT
SOL	96.8 ±8.6	107.9 ±7.7	138.3 ±9.4**	127.4 ±11.0*
EDL	92.3 ±9.3	142.4 ±8.1**	133.3 ±10.3*	111.1 ±10.0
GAST	81.8 ±9.1	117.0 ±4.5*	130.9 ±5.0**	117.1 ±10.0**

Table 3: Protein Degradation

	NPD	IGF	IGF+HPD	CONT
3Me-histidine (umol/ml*100g b.w.):				
	1.18 ±.18	.567 ±.08**	.619 ±.11**	.466 ±.04**
3Me-histidine (umol/mg creatinine):				
	.284 ±.01	.186 ±.02	.209 ±.02	.296 ±.03
3Me-histidine (umol/ml):				
	.168 ±.02	.129 ±.03	.115 ±.01	.139 ±.02

Table 4: DNA Content (ug/mg muscle)

	NPD	IGF	IGF+HPD	CONT
SOL	4.46 ±.45	4.38 ±.60	5.17 ±.59	2.93 ±.25*
EDL	3.28 ±.51	3.67 ±.46	4.19 ±.30	1.62 ±1.8**
GAST	4.10 ±.49	5.06 ±.42	3.90 ±.21	1.38 ±.16**

Table 5: RNA Content (ug/mg muscle)

	NPD	IGF	IGF+HPD	CONT
SOL	5.80 ±.50	4.32 ±.43	6.13 ±.56	4.41 ±.46
EDL	4.21 ±.45	4.42 ±.29	4.55 ±.57	2.59 ±.26**
GAST	5.65 ±.53	5.51 ±.39	5.75 ±.58	2.79 ±.16**

Table 6: Endurance (seconds)

Week	NPD	IGF	IGF+HPD	CONT
1	27.1 ±.2.9	25.3 ±3.5	26.7 ±4.1	-
2	23.9 ±3.5	28.6 ±4.3	37.4 ±5.5	
3	23.8 ±3.6	34.1 ±4.6	30.2 ±7.3	
4	24.6 ±4.0	35.3 ±6.0	39.3 ±6.2	

Table 7: Hind Limb Utilization (5=best, 1=worst)

Week	NPD	IGF	IGF+HPD	CONT
1	3.40 ±.24	3.68 ±.09	3.20 ±.19	5.0
2	2.85 ±.22	3.80 ±.20**	3.48 ±.17	
3	2.54 ±.17	3.51 ±.11**	3.34 ±.12**	
4	2.60 ±.13	3.40 ±.15**	3.35 ±.12**	

These data are also presented graphically in the Figures.

As shown in Figures 2-4, and 6, the increased protein synthesis is accompanied by increased muscle content of DNA, RNA and protein. At the same time, muscle degradation, as measured by urinary 3-methylhistidine excretion, is decreased (Figure 5). Functionally, dystrophic mice treated with hIGF-I showed improvement in muscle endurance and hind limb utilization (Figure 1). When hIGF-I is combined with high protein diet, protein synthesis in soleus, EDL and gastrocnemius muscle is further enhanced (Figure 4), while protein degradation is decreased below that of hIGF-I alone (Figure 5). This combination therapy also causes additional increases in nucleic acid and protein content of dystrophic muscle (Figure 2, 3 and 6), with additional enhancement of muscle endurance and limb flexibility (Figure 1). As seen in Figure 6, net muscle protein content was significantly increased (significant at .01 level) for hIGF and high protein diet in soleus muscle and gastrocnemius muscle and for hIGF alone in EDL muscle. Figure 6 also shows that net muscle protein content was significantly increased (significant at .05 level) for hIGF and high protein diet in soleus muscle and for IGF alone in gastrocnemius muscle. In addition, as expected hIGF-I, high protein diet and the combination of the two stimulated weight gain and growth in dystrophic mice. In summary, 4 week administration of hIGF-I in the presence of a high protein diet has resulted in both biochemical and functional improvement in dystrophic mice.

For Figures 1-6, n = 10 to 14 animals per group. All values are expressed as mean ± s.e.m. Statistical comparisons of treated groups are made versus those of untreated mice. (HPD = high protein diet, dy = dystrophic mice).

Figure 7 shows muscle fiber from a dystrophic mouse, a dystrophic mouse given a high protein diet, a non-dystrophic mouse, and a dystrophic mouse given hIGF-1. In the non-dystrophic mouse, the muscle fibers have a more uniform diameter, and there is no evidence of fiber necrosis or extra fiber spaces. In contrast, the dystrophic control mouse had non-uniform muscle fiber diameters, showed fiber necrosis and extra fiber spaces. The muscle fiber from both a dystrophic mouse given either hIGF-1 or a high protein diet were more like that seen in the non-dystrophic mouse than in the dystrophic control mouse.

Example C - Hamsters

A study is undertaken to examine the effect of hIGF-I on the biochemical, morphologic and muscle function of dystrophic hamsters. In particular, the effect of hIGF-I on muscle protein synthesis and protein degradation is examined. Also examined is whether high protein diet and/or daily submaximal exercise enhance the effect of hIGF-I.

A preliminary study involving 12 BIO 53.58 dystrophic hamsters is carried out to determine the most appropriate dose of hIGF-I that is well tolerated and does not cause significant

hypoglycemia. After the proper dose is calculated, the hamsters, which total seventy-two in number, are split into groups as set out below, with twelve hamsters in each group.

Phase I

Group 1 - dystrophic hamsters receiving normal diet (20% protein).

Group 2 - dystrophic hamsters receiving hIGF-I with normal diet.

Group 3 - dystrophic hamsters receiving high protein diet (50% protein).

Group 4 - dystrophic hamsters receiving submaximal exercise with normal diet.

Group 5 - non dystrophic, control hamsters receiving normal diet.

Phase II

Group 1 - dystrophic hamsters receiving normal diet.

Group 2 - dystrophic hamsters receiving hIGF-I with high protein diet.

Group 3 - dystrophic hamsters receiving hIGF-I with normal diet and submaximal exercise.

Group 4 - dystrophic hamsters receiving hIGF-I with high protein diet and submaximal exercise.

Group 5 - dystrophic hamsters receiving high protein diet and submaximal exercise.

Group 6 - non dystrophic, control hamsters receiving normal diet.

The hamsters are received at 60 days of age, with the control animals matched for age and sex. All of the hamsters remain on their respective protocols for eight weeks. The hamsters receive twice daily subcutaneous injections of hIGF-I (8 am and 5 pm) into the skin fold at the back of the neck. All

of the hamsters have free access to both food and water. The normal diet consists of 20% protein while the high protein diet contains 50% protein. Submaximal exercise therapy consists of daily twenty minute periods on a motorized rotary treadmill at a slow speed of 4 meters/minute. Exercise is terminated if dystrophic hamsters show signs of fatigue such as dragging of their legs and falling.

Blood for glucose, insulin, c-peptide and amino acids is drawn from the suborbital sinus prior to starting therapy, at 4 weeks, and at the end of eight weeks. Weekly measures of weight and length are made in all animals.

To determine protein synthesis and degradation, at the end of eight weeks on their respective treatments, the in vivo measurement of protein synthesis and degradation is made via a hemicorpus perfusion of the lower limbs carried out according to the method of Jefferson et al., A technique for perfusion of an isolated preparation of rat hemicorpus, Methods Enzymol. 39:73-82 (1975) and Li, Protein synthesis and degradation in skeletal muscle of normal and dystrophic hamsters, Am. J. Physiol. 239:401-406 (1980). All references mentioned herein are incorporated by reference. Protein synthesis is calculated from the nanomoles of labelled phenylalanine incorporated into muscle protein. Protein degradation is determined from the dilution of ^{14}C-phenylalanine by ^{12}C-phenylalanine released from muscle protein. Release of 3-methylhistidine into the perfusion medium is used as another indicator of muscle protein degradation.

Urine is removed directly from the bladder for measurement of urinary 3-methylhistidine excretion and creatinine.

Biochemical analysis of soleus, EDL and gastrocnemius muscles for DNA, RNA, free amino acids, ATP, glycogen, cathepsin B, and cathepsin D, will be obtained at the termination of treatment.

Muscle endurance is evaluated by the length of time the animals remain suspended from a wire rack 12 inches above the table top. The test is terminated when the animal exhibits signs of fatigue or drops onto the cushion below. All animals are tested prior to starting the protocol and once per week for the remainder of the protocol.

To analyze the morphology of the hamsters, post treatment samples of soleus, EDL and gastrocnemius muscle are fixed in formalin and isotonic glutaraldehyde for examination by light and electron microscopy respectively. The percent of necrotic fibers, abnormalities in the distribution of fiber sizes, inflammatory cell number and satellite cell number are determined by light microscopy. Plasma membrane alternations, basal lamina replication, mitochondrial swelling or degeneration, as well as, decreases in myofilament number, fiber rarefaction, hypercontraction of fibers, ghost fibers and dilation of the sarcoplasmic reticulum is determined by electron microscopy.

CLAIMS

1. A method of treating a muscle disorder, which demonstrates 1) a decrease in muscle protein synthesis, 2) increase in protein degradation or 3) both comprising administering to a mammal previously identified as having said condition an effective dosage of IGF-I.

2. The method according to claim 1, wherein said muscle condition is selected from the group consisting of myotonic dystrophy, a multisystemic, autosomal dominant disorder associated with progressive muscle wasting, and manifested by hyperinsulinemia and insulin resistance.

3. The method according to claim 1, wherein said muscle condition is selected from the group consisting of Duchenne muscle dystrophy, Becker muscle dystrophy, fascio scapular humoral dystrophy, limb girdle dystrophy, acid maltase deficiency and nemaline myopathy.

4. The dosage of the method according to claim 1, further comprising administering said dosage at least twice daily.

5. In the method according to claim 1, said effective dose of IGF-I produces an anabolic effect, but is insufficient to cause hypoglycemia.

6. The method according to claim 5, further comprising preparing said dosage of about 0.06 to about 0.12 mg/kg/dose of IGF-I.

7. The method according to claim 1, further comprising measuring the mammal's blood glucose level periodically, and if said blood glucose level is less than about 60 mg/dl, reducing the dose of IGF-I to less than about 0.06 mg/dl.

8. The method according to claim 1, wherein said administration comprises a subcutaneous injection.

9. The method according to claim 1, further comprising daily administration of said effective dose for an extended period.

10. The method according to claim 1, further comprising putting the mammal on a high protein diet.

11. The method according to claim 10, further comprising continuing the mammal on said diet through substantially the entire treatment period.

12. The method according to claim 1, further comprising submaximal exercise of the mammal at least three times a week during said IGF-I treatment period.

13. In the method according to claim 1, said IGF-I consisting essentially of hIGF-I produced by a non-human organism harboring and expressing the gene for hIGF-I.

14. The method according to claim 1, wherein said mammal is livestock.

15. The method according to claim 1, wherein said mammal is a canine.

16. The method according to claim 1, wherein said mammal is a human.

17. The method according to claim 16, further comprising, at least about eight days before treatment with IGF-I, putting said human on a diet of approximately 47 kcal kg^{-1} d^{-1}, including a protein intake of greater than 2 g kg^{-1} d^{-1}.

18. The diet of the method according to claim 16, consisting essentially of approximately 45% carbohydrate, 25% protein and 30% fat of daily caloric requirements.

19. The method according to claim 16, further comprising continuing the human on said diet through substantially the entire treatment period.

20. The method according to claim 1, wherein said muscle condition is myotonic dystrophy.

21. A method of treating muscular dystrophy comprising administering to a mammal previously identified as having said disorder an effective dosage of IGF-I.

22. A method of treating a muscle disorder which demonstrates 1) a decrease in muscle protein synthesis, 2) an increase in muscle protein degradation or 3) both comprising administering to a mammal previously identified as having said condition a daily dosage of about 0.06 to about 0.12 mg/kg/dose of IGF-I for an extended period, combined with a high protein diet and submaximal exercise of the mammal at least three times a week during said IGF-I treatment period.

23. A method of treating a muscle disorder, which demonstrates muscle wasting or weakness comprising administering to a mammal previously identified as having said condition an effective dosage of IGF-I.

FIG. 1A

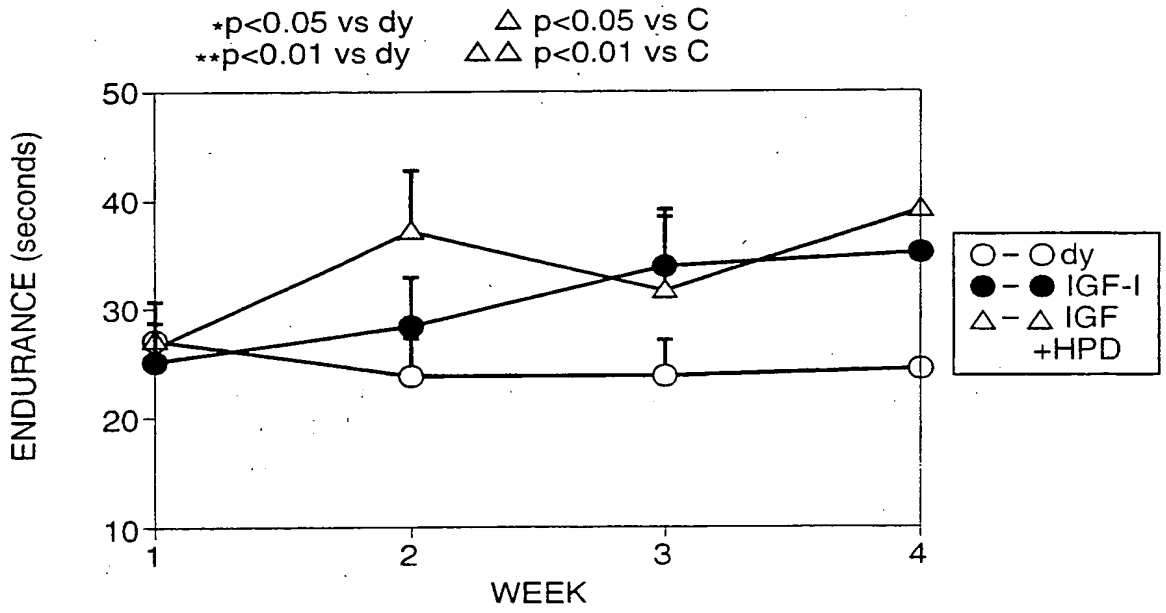
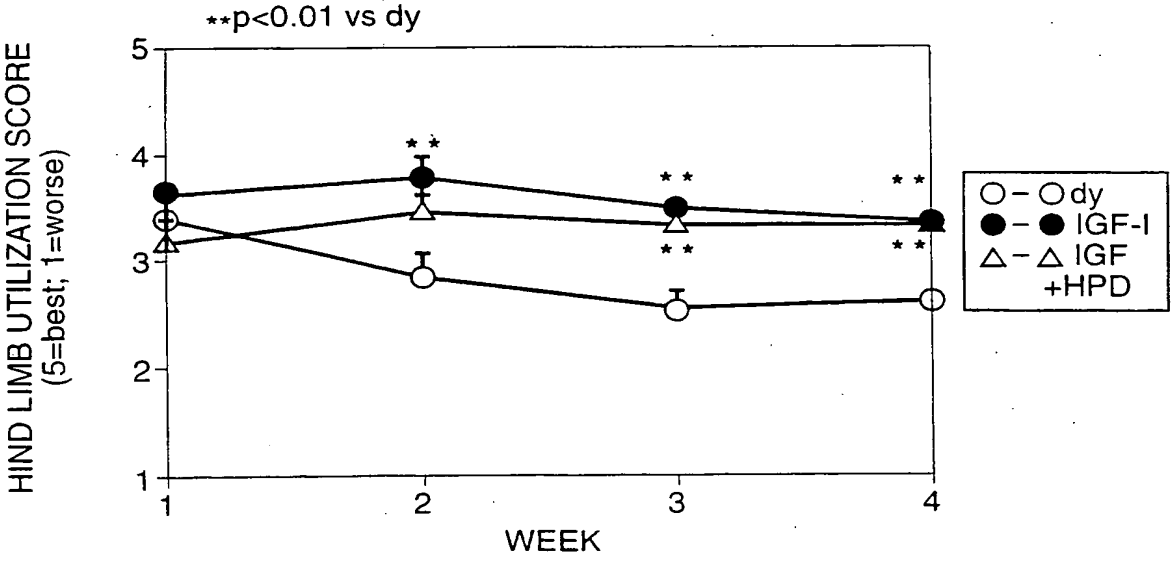


FIG. 1B



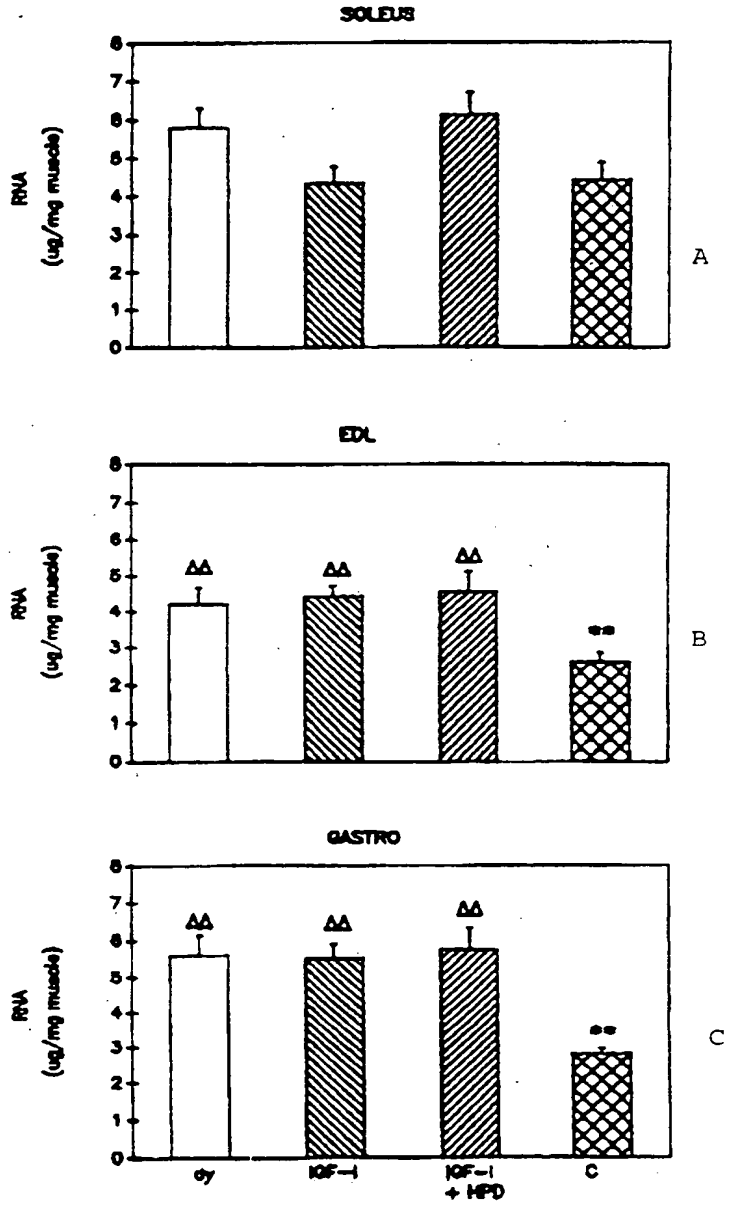

SOLEUS
RNA (ug/mg muscle)
A
EDL
RNA (ug/mg muscle)
ΔΔ
**
B
GASTRO
RNA (ug/mg muscle)
ΔΔ
**
C
Cy
IGF-I
IGF-I + HPD
C

Fig. 2

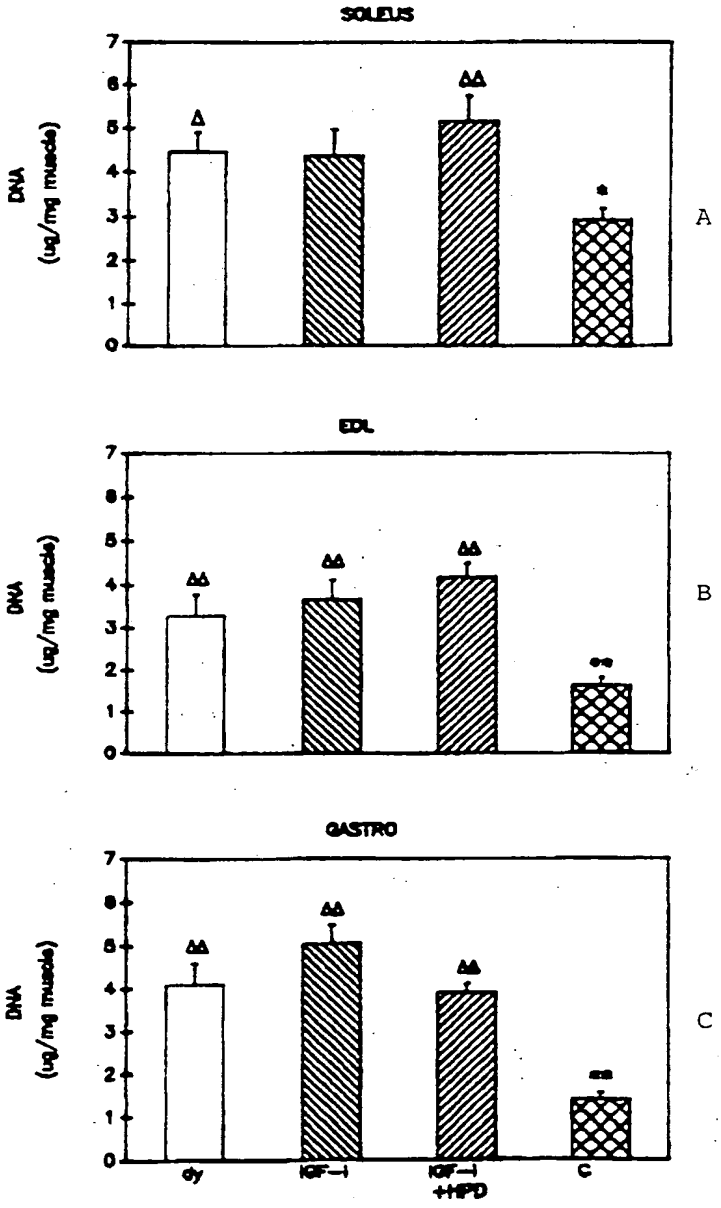

SOLEUS
DNA (ug/mg muscle)
A
EDL
DNA (ug/mg muscle)
B
GASTRO
DNA (ug/mg muscle)
C
Gly
IGF-I
IGF-I +HPD
C

Fig. 3

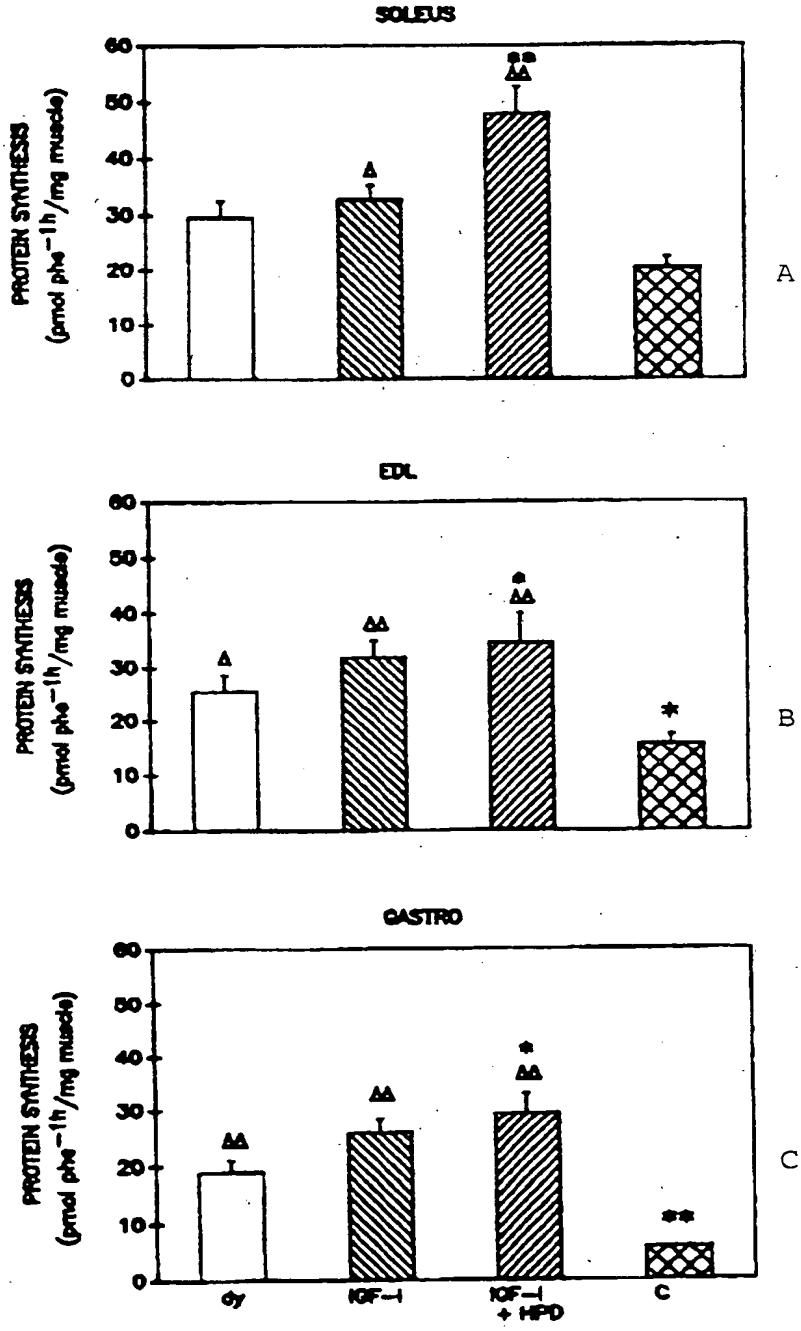

SOLEUS
PROTEIN SYNTHESIS (pmol phe⁻¹h/mg muscle)
0
10
20
30
40
50
60
A
EDL
PROTEIN SYNTHESIS (pmol phe⁻¹h/mg muscle)
B
GASTRO
PROTEIN SYNTHESIS (pmol phe⁻¹h/mg muscle)
C
dy
IGF-I
IGF-I + HFD
C

Fig. 4

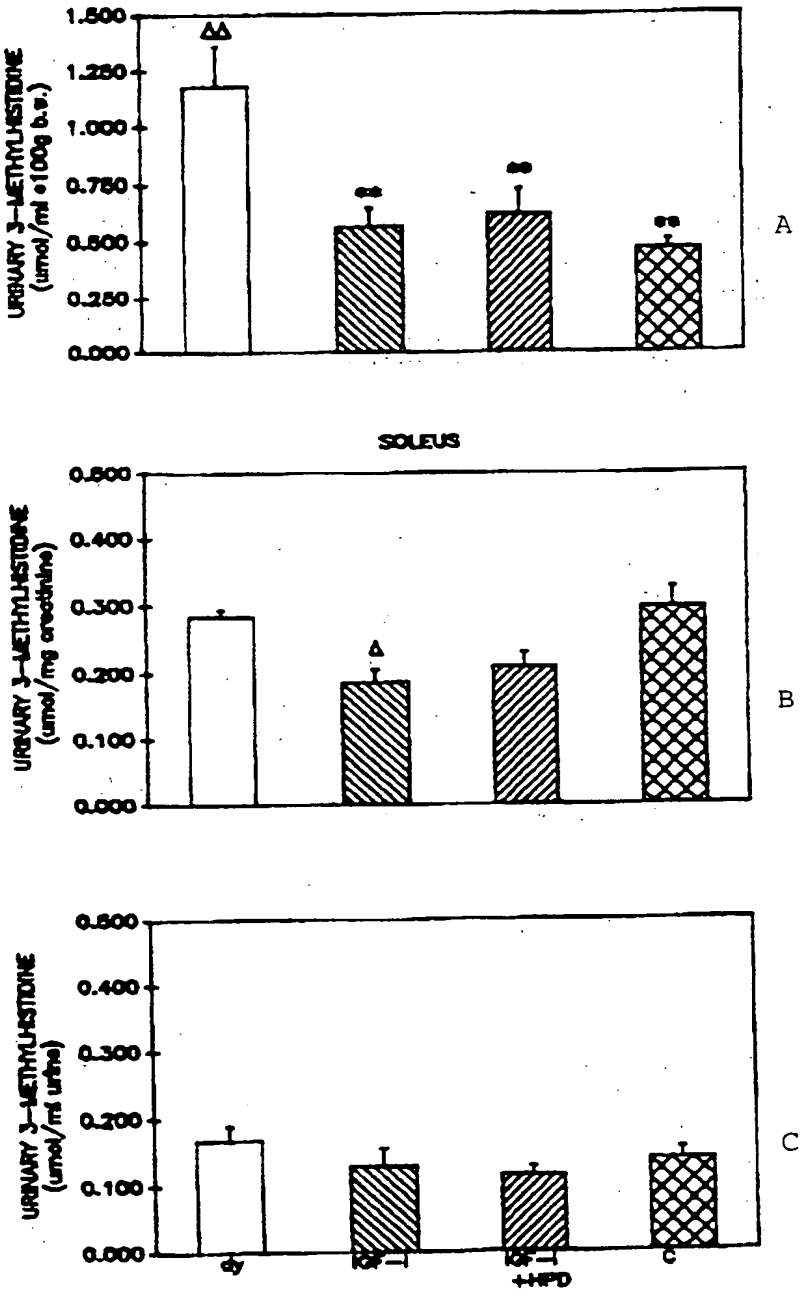

URINARY 3-METHYLHISTIDINE (umol/ml • 100g b.w.)
1.500
1.250
1.000
0.750
0.500
0.250
0.000
ΔΔ
••
••
••
A
SOLEUS
URINARY 3-METHYLHISTIDINE (umol/mg creatinine)
0.500
0.400
0.300
0.200
0.100
0.000
Δ
B
URINARY 3-METHYLHISTIDINE (umol/ml urine)
0.500
0.400
0.300
0.200
0.100
0.000
dy
IGF-I
IGF-I +HPD
C
C

Fig. 5

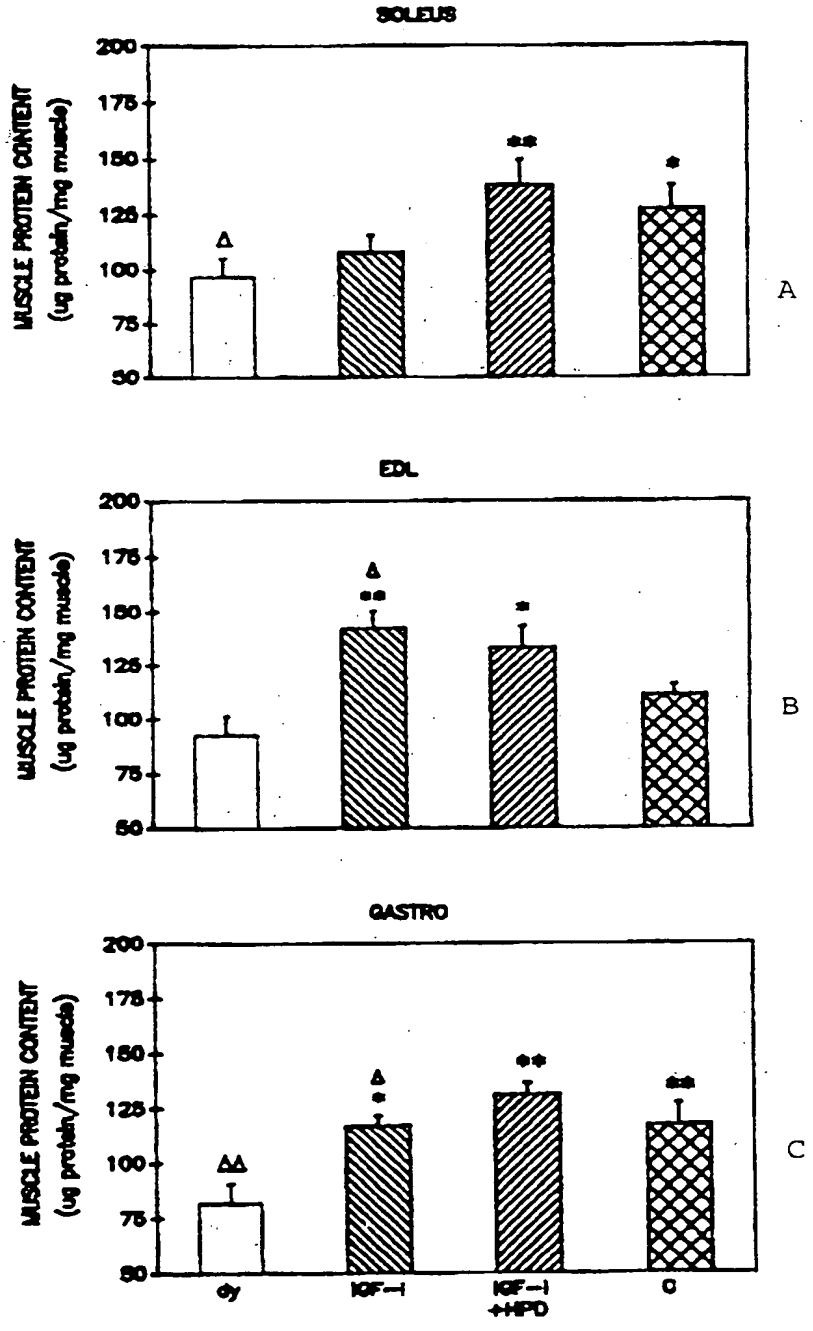

SOLEUS
MUSCLE PROTEIN CONTENT (ug protein/mg muscle)
200
175
150
125
100
75
50
Δ
**
*
A
EDL
MUSCLE PROTEIN CONTENT (ug protein/mg muscle)
200
175
150
125
100
75
50
Δ
**
*
B
GASTRO
MUSCLE PROTEIN CONTENT (ug protein/mg muscle)
200
175
150
125
100
75
50
ΔΔ
Δ
*
**
**
dy
IGF-I
IGF-I +HPD
C
C

Fig. 6

FIG. 7A

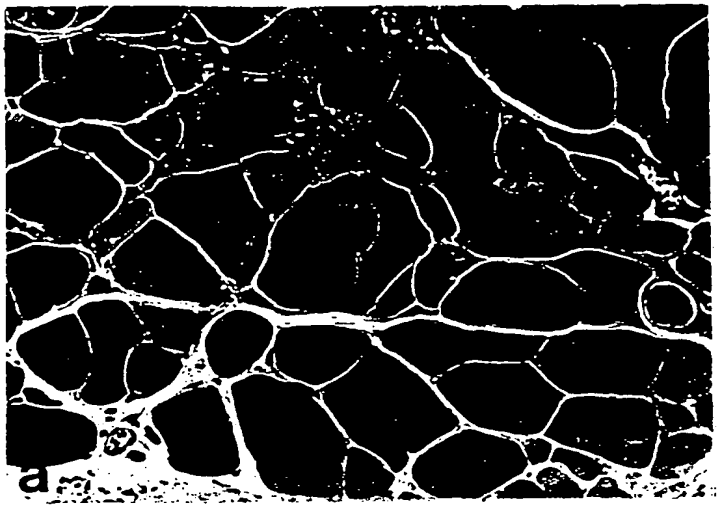
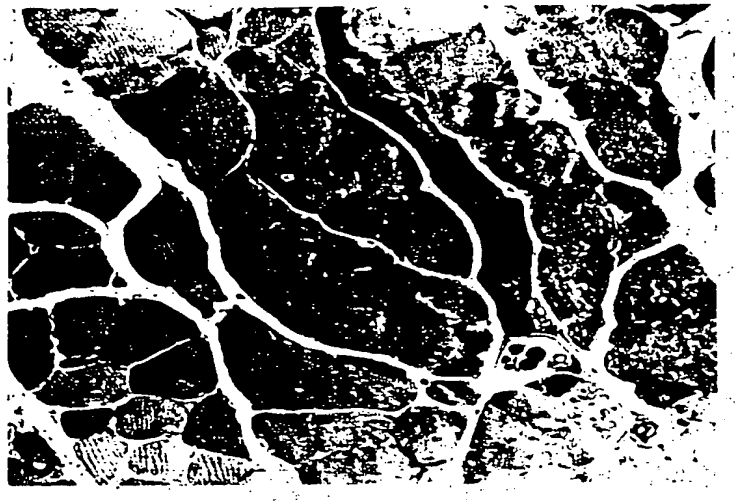


FIG. 7B



RECTIFIED SHEET (RULE 91)

FIG. 7C

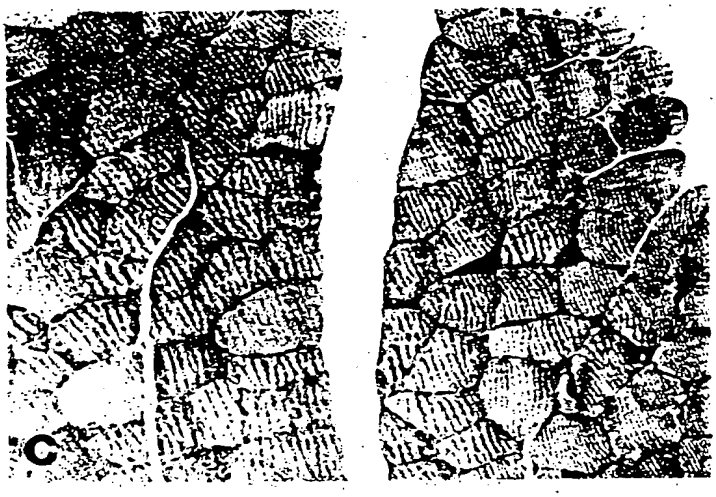
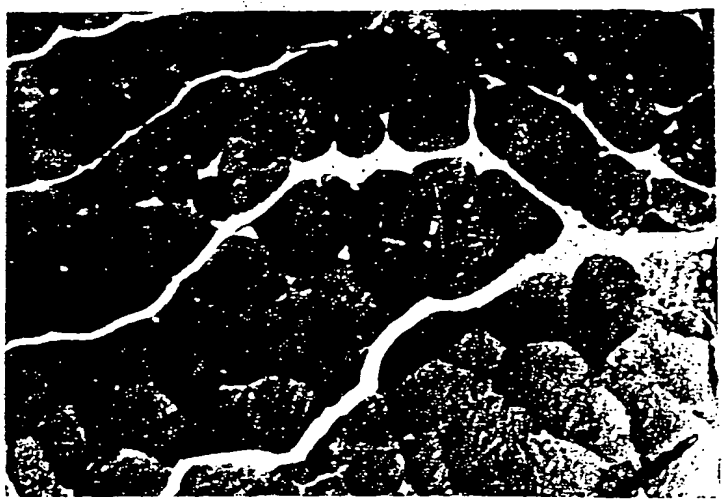


FIG. 7D



INTERNATIONAL SEARCH REPORT

Int. ..ational application No.
PCT/US94/13137

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07K 14/00; A61K 38/27
US CL : 514/12, 21
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A, 90/15142 (WELLS ET AL.) 13 December 1991, see entire document.	1-23
X	WO, A, 90/03154 (FRYKLUND ET AL.) 05 March 1992, see entire document.	1-23
X	US, A, 5,077,276 (BALLARD ET AL.) 31 December 1990, see entire document.	1-23

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 JANUARY 1995	Date of mailing of the international search report 09 MAR 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer P. LYNN TOUZEAU, PH.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*